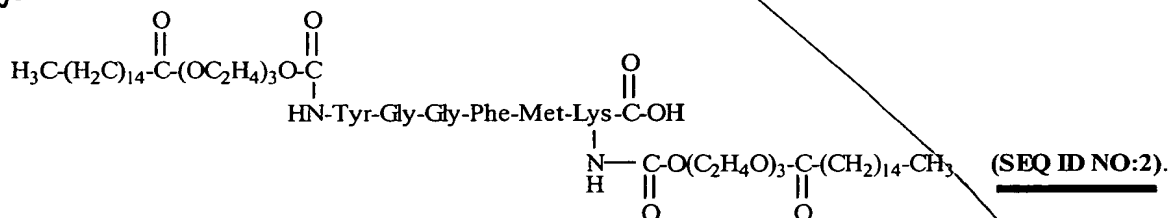


97. (Amended) The method of claim 46 wherein the drug-oligomer conjugate has a formula:



#### REMARKS

The amendments above conform the specification and claims to the requests contained in the Office Action mailed January 18, 2001. Marked-up versions of the substituted paragraphs are provided at Appendix A.


Applicants have also taken this opportunity to correct various typographical errors in the specification. For example, at page 25, line 1, the term "Trp-Pro-Lys-His-Xaa-NH<sub>2</sub>" has been replaced with the term --Trp-Trp-Pro-Lys-His-Xaa-NH<sub>2</sub>-- in order to correct a typographical error. If required, support for this amendment can be found at column 1, line 66-67 of U.S. Patent No. 5,641,861, which was incorporated by reference in the present application.

Applicants have also amended Figures 2-8 to reference sequence ID numbers as requested by the Examiner. Amended Figures 2-8 are enclosed herein.

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Any questions regarding the foregoing should be directed to the undersigned, who may be reached at (919) 854-1400.

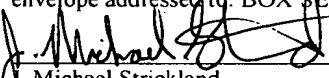
Respectfully submitted,

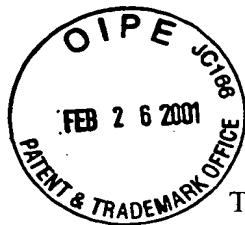
  
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J. Michael Strickland  
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## APPENDIX A

### Marked-Up Replacement Paragraphs

The paragraph beginning at page 24, line 28 has been amended as follows:

In another [other] aspect, the therapeutic peptide of the amphiphilic drug-oligomer conjugates are as described in United States Patent 5,641,861, which is incorporated herein by reference, so long as any of such peptides contains a lysine residue. Exemplary peptides described therein include: Ac-Phe-Arg-Trp-Trp-Tyr-Lys—NH<sub>2</sub> (SEQ ID NO:4); Ac-Arg-Trp-Ile-Gly-Trp-Lys—NH<sub>2</sub> (SEQ ID NO:5); Trp-Trp-Pro-Lys-His-Xaa—NH<sub>2</sub> (SEQ ID NO:6), where Xaa can be any one of the twenty naturally occurring amino acids, or Trp-Trp-Pro-Xaa—NH<sub>2</sub> (SEQ ID NO:7), where Xaa is Lys or Arg; Tyr-Pro-Phe-Gly-Phe-Xaa—NH<sub>2</sub> (SEQ ID NO:8), wherein Xaa can be any one of the twenty naturally occurring amino acids; (D)Ile-(D)Met-(D)Ser-(D)Trp-(D)Trp-Gly<sub>n</sub>-Xaa—NH<sub>2</sub> (SEQ ID NO:9), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid and n is 0 or 1, peptides of this formula can be hexapeptides when Gly is absent (n is 0) and heptapeptides when Gly is present (n is 1); (D)Ile-(D)Met-(D)Thr-(D)Trp-Gly-Xaa—NH<sub>2</sub> (SEQ ID NO:10), wherein Xaa is Gly or the D-form of a naturally-occurring amino acid; Tyr-A1-B2-C3—NH<sub>2</sub> (SEQ ID NO:11), wherein A1 is (D)Nve or (D)Nle, B2 is Gly, Phe, or Trp, and C3 is Trp or Nap; Pm and red {Me<sub>x</sub>H<sub>y</sub>N-Tyr-(NMe)<sub>z</sub>-Tyr-Xaa<sub>z</sub>—NH<sub>2</sub>} (SEQ ID NO:12), wherein x and y independently are 0,1, or 2 and z is 0 or 1, and wherein Xaa is Phe, D-Phe, or NHBzl; Trp-Trp-Pro-D4-His<sub>z</sub>-Xaa<sub>z</sub>-NH<sub>2</sub> (SEQ ID NO:13), wherein z is 0 or 1, D4 is Lys or Arg and Xaa is any one of the naturally-occurring amino acids.

The paragraph beginning at page 25, line 12 has been amended as follows:

In still another [other] aspect, the therapeutic peptide of the amphiphilic drug-oligomer conjugates are as described in United States Patent 5,602,099, which is incorporated herein by reference. with the proviso that the conjugation can occur only where there is a free carboxyl or free N-terminal. Exemplary peptides include: H-Tyr-Tic-Phe-Phe-OH (SEQ ID NO:14); H-Tyr-Tic-Phe-Phe-NH<sub>2</sub> (SEQ ID NO:15); Tyr(NaMe)-Tic-Phe-Phe-OH (SEQ ID

NO:16); Tyr(NaCpm)-Tic-Phe-Phe-OH (SEQ ID NO:17); Tyr(NaHex)-Tic-Phe-Phe-OH (SEQ ID NO:18); Tyr(NaEt<sub>2</sub>)-Tic-Phe-Phe-OH (SEQ ID NO:19); H-Dmt-Tic-Phe-Phe-OH (SEQ ID NO:20); H-Dmt-Tic-Phe-Phe-NH<sub>2</sub> (SEQ ID NO:21); H-Tyr(3-F)-Tic-Phe-Phe-OH (SEQ ID NO:22); H-Tyr(3-Cl)-Tic-Phe-Phe-OH (SEQ ID NO:23); H-Tyr(3-Br)-Tic-Phe-Phe-OH (SEQ ID NO:24); H-Dmt-TicΨ[CH<sub>2</sub>—NH]Phe-Phe-OH (SEQ ID NO:25); H-Dmt-TicΨ[CH<sub>2</sub>—NH]Phe-Phe-NH<sub>2</sub> (SEQ ID NO:26); H-Tyr-TicΨ[CH<sub>2</sub>—NCH<sub>3</sub>]Phe-Phe-OH (SEQ ID NO:27); H-Tyr-TicΨ[CH<sub>2</sub>—NH]Hfe-Phe-OH (SEQ ID NO:28); Tyr(NMe)-TicΨ[CH<sub>2</sub>—NH]Hfe-Phe-OH (SEQ ID NO:29); H-Tyr-Tic-Phg-Phe-OH (SEQ ID NO:30); H-Tyr-Tic-Trp-Phe-OH (SEQ ID NO:31); H-Tyr-Tic-Trp-Phe-NH<sub>2</sub> (SEQ ID NO:32); H-Tyr-Tic-His-Phe-OH (SEQ ID NO:33); H-Tyr-Tic-2-Nal-Phe-OH (SEQ ID NO:34); H-Tyr-Tic-Atc-Phe-OH (SEQ ID NO:35); H-Tyr-Tic-Phe-Phe(pNO<sub>2</sub>)-OH (SEQ ID NO:36); H-Tyr-Tic-Trp-Phe(pNO<sub>2</sub>)-OH (SEQ ID NO:37); H-Tyr-Tic-Phe-Trp-NH<sub>2</sub> (SEQ ID NO:38); H-Tyr-Tic-Phe-Phe-Val-Val-Gly-NH<sub>2</sub> (SEQ ID NO:39); H-Tyr-Tic-Phe-Phe-Tyr-Pro-Ser-NH<sub>2</sub> (SEQ ID NO:40); H-Tyr-Tic-Trp-Phe-Tyr-Pro-Ser-NH<sub>2</sub> (SEQ ID NO:41); H-Tyr-Tic-Trp-Phe (pNO<sub>2</sub>) -Tyr-Pro-Ser-NH<sub>2</sub> (SEQ ID NO:42) and H-Tyr-Tic-Phe-Phe-Leu-Nle-Asp-NH<sub>2</sub> (SEQ ID NO:43).

The paragraph beginning at page 25, line 27 has been amended as follows:

Abbreviations in the aforementioned peptides of U.S. Patent 5,602,099 may be interpreted as follows: Aib=α-aminoisobutyric acid; Atc=2-aminotetralin-2-carboxylic acid; Boc=tert-butoxycarbonyl; Cpm=cyclopropylmethyl; DCC=dicyclohexyl-carbodiimide; DIEA=diisopropylethylamine; Dmt=2,6-dimethyltyrosine; Et=ethyl; Hex=hexyl; Hfe=homophenylalanine; HOBt=1-hydroxybenzotriazole; MVD=mouse vas deferens; 1-Nal=3-(1'-naphthyl)alanine; 2-Nal=3-(2'-naphthyl)alanine; Phe(pNO<sub>2</sub>)=4-nitrophenylalanine; Phg=phenylglycine; Tic=1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TIP=H-Tyr-Tic-Phe-OH (SEQ ID NO:44); TIP-NH<sub>2</sub>=H-Tyr-Tic-Phe-NH<sub>2</sub> (SEQ ID NO:45); TIP(Ψ)=H-Tyr-TicΨ[CH<sub>2</sub>-NH]Phe-OH (SEQ ID NO:46); TIPP=H-Tyr-Tic-Phe-Phe-OH (SEQ ID NO:14); TIPP-NH<sub>2</sub>=H-Tyr-Tic-Phe-Phe-NH<sub>2</sub> (SEQ ID NO:15); TIPP(Ψ)=H-Tyr-TicΨ[CH<sub>2</sub>-NH]Phe-Phe-OH (SEQ ID NO:47); Tyr(3-Br)=3-bromotyrosine; Tyr(3-Cl)=3-chlorotyrosine; Tyr(3-F)=3-fluorotyrosine; and Tyr(NaMe)=Na-methyltyrosine.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

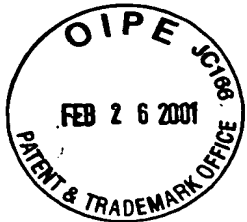
In re: Ekwuribe et al.

Serial No.: 09/430,735

Filed: 29 October 1999

For: *METHODS OF INDUCING ANALGESIA*

February 20, 2001



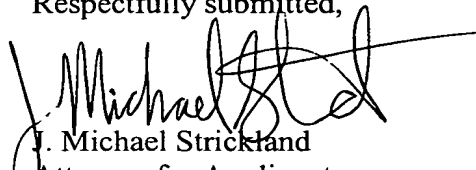
STATEMENT IN SUPPORT OF FILING A  
SEQUENCE LISTING UNDER 37 CFR § 1.821(f)

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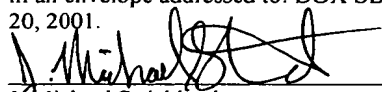
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# Stability of Enkephalin and Cetyl-PEG<sub>2</sub>-Enkephalin in Rat Brain Homogenate

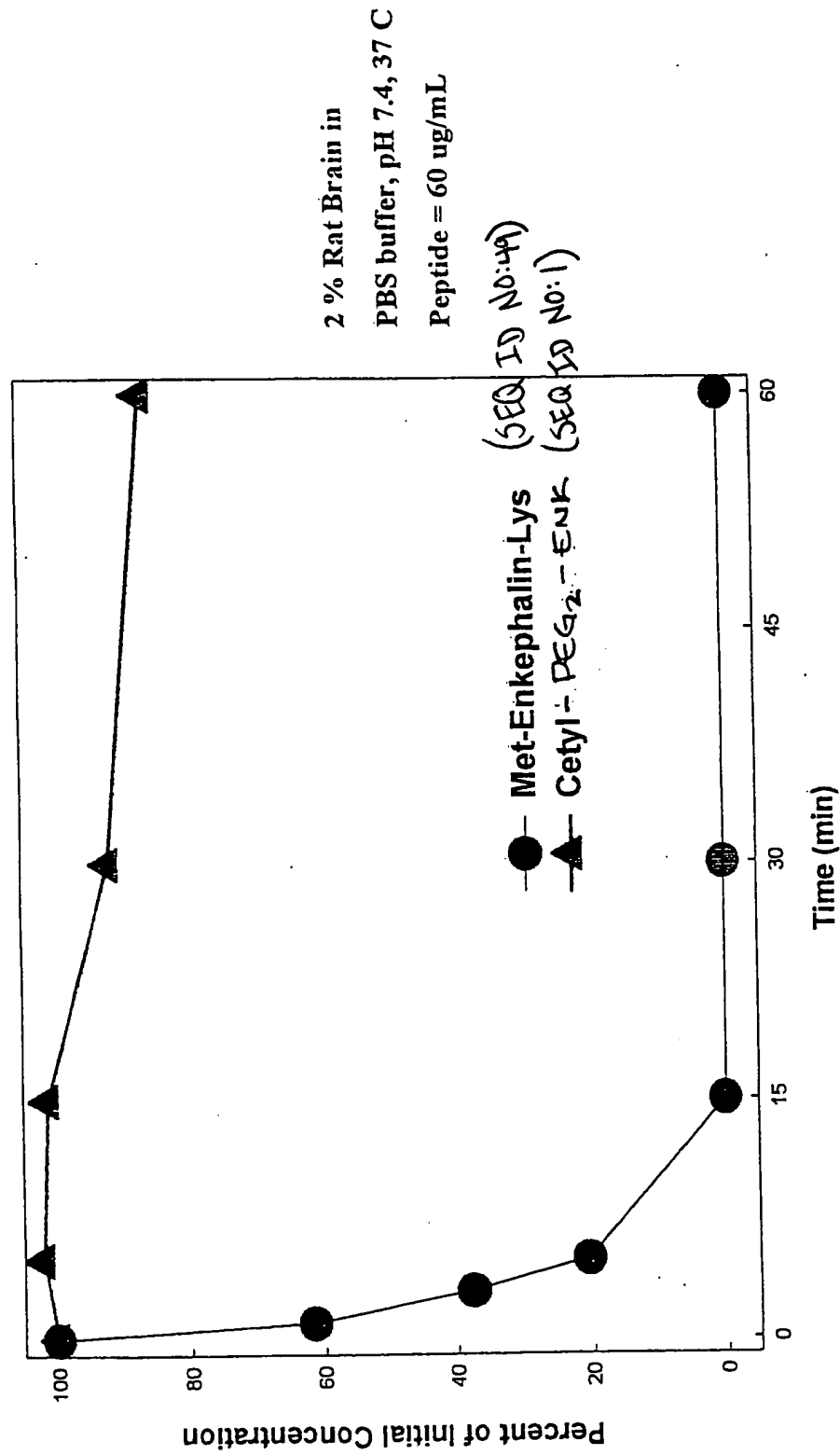


FIGURE 2

# Stability of Cetyl-PEG<sub>2</sub> Enkephalin in Rat Brain Homogenate

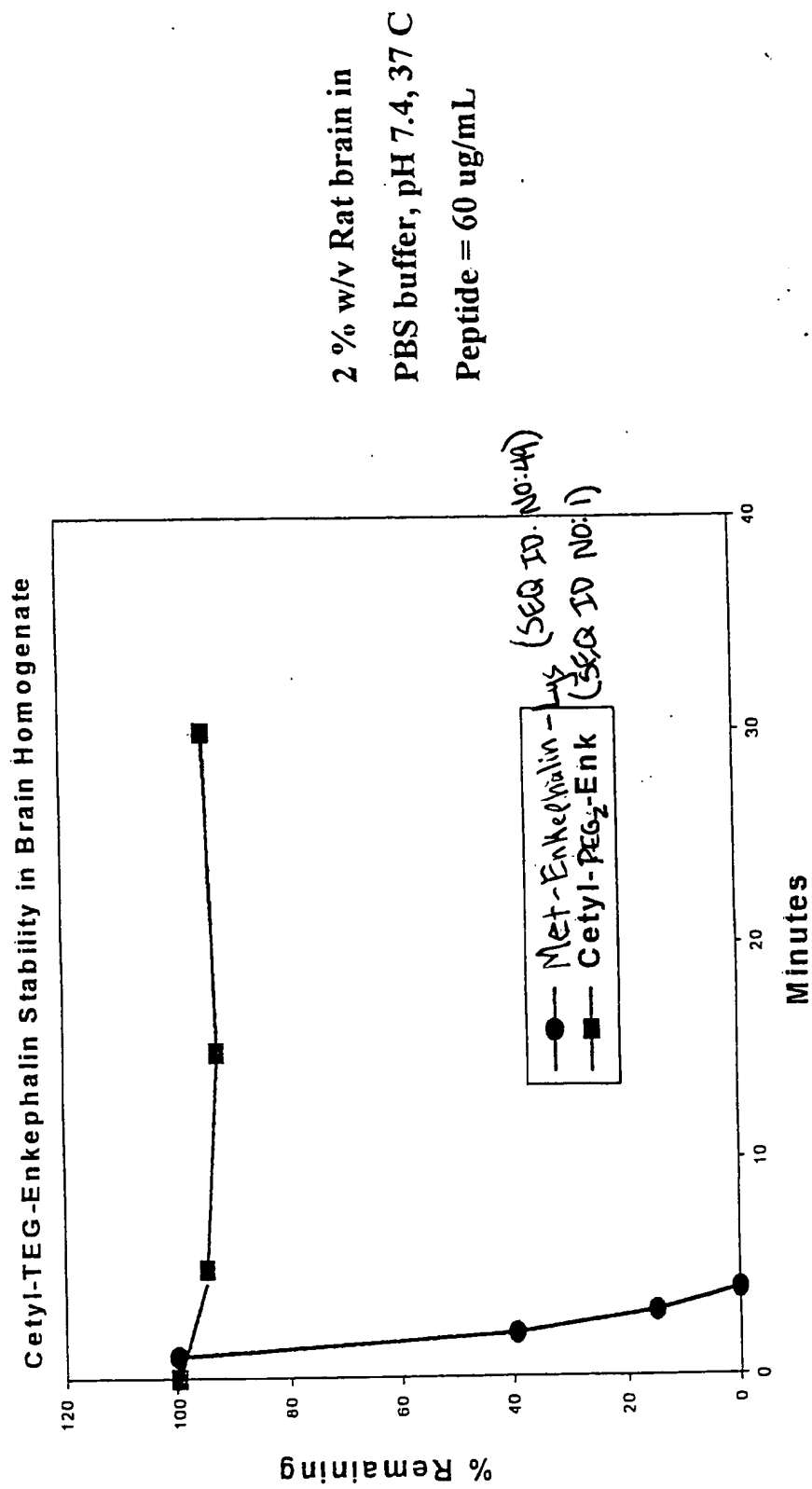
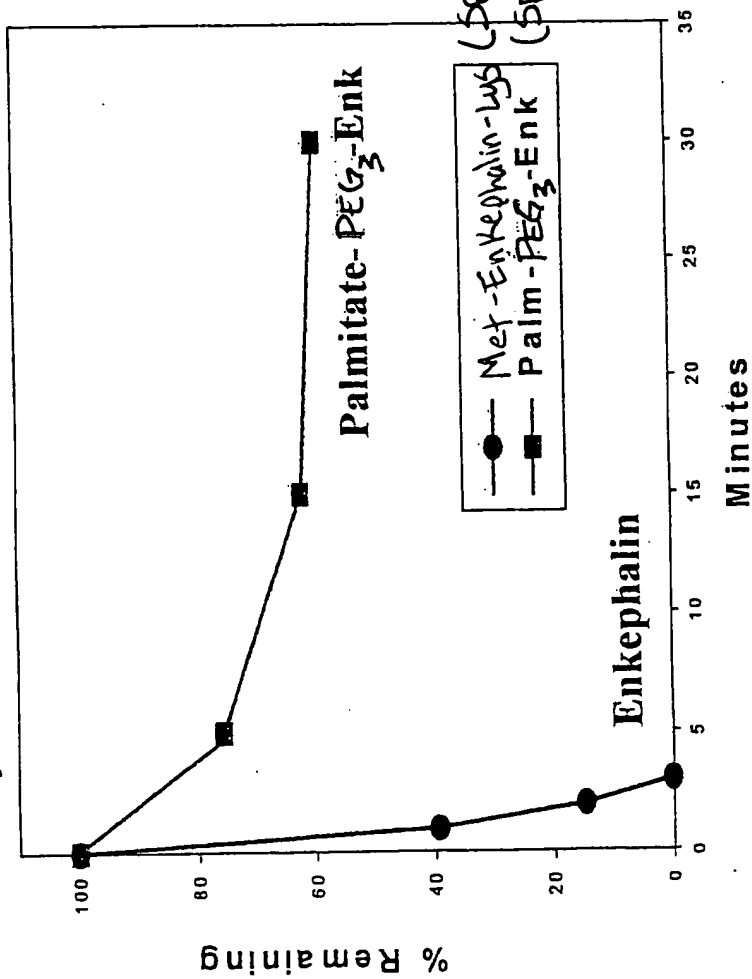


FIGURE 3

# Stability of Palmitate-PEG<sub>3</sub>-Enkephalin in Rat Brain Homogenate

Stability of Palmitate-TEG-Enk in Brain homogenate



2% w/v Rat Brain in  
PBS buffer, pH 7.4, 37 C  
Peptide = 60ug/mL

(SEQ ID No:14)  
(SEQ ID No:1)

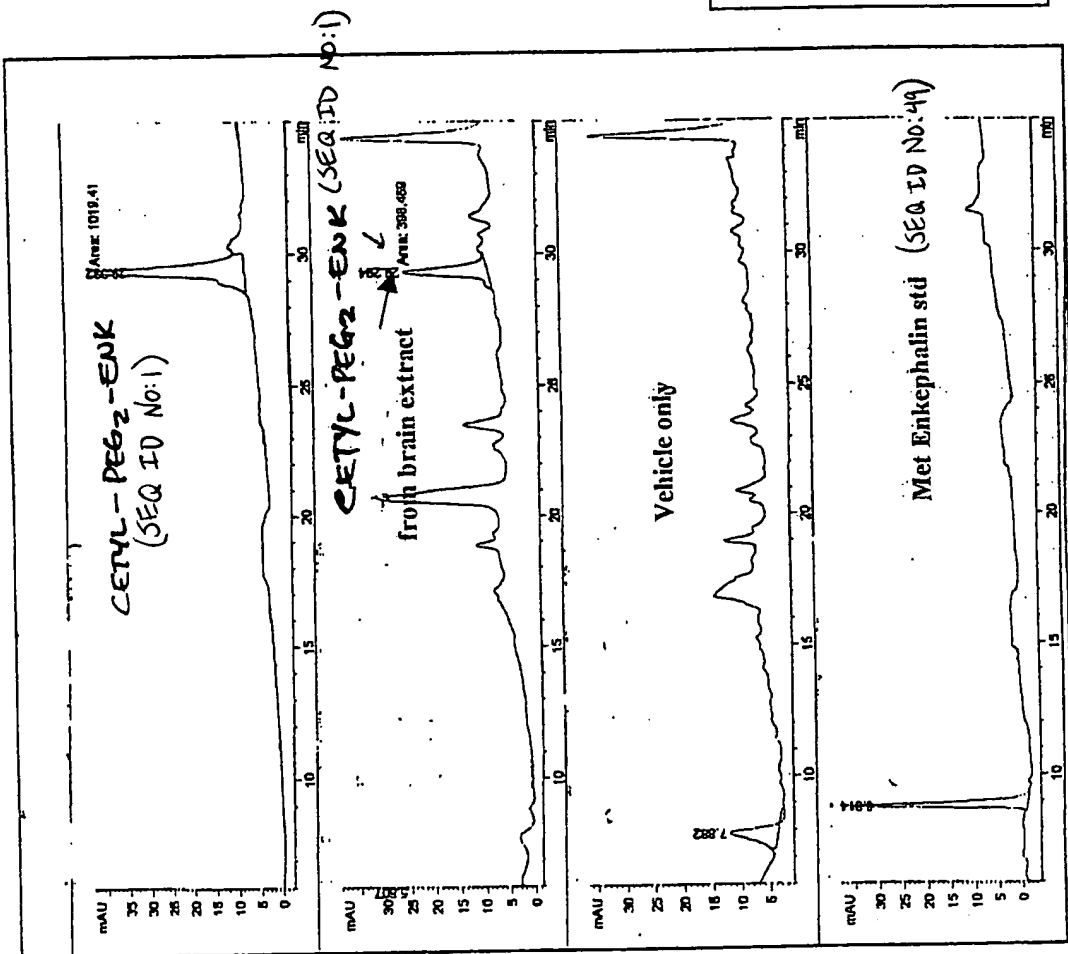
FIGURE 4



(SEQ ID No:1)

# Isolation of Cetyl-PEG<sub>2</sub>-Enkephalin from the Brain

FIGURE 5A



HPLC conditions:  
 Column: C-18  
 Solvent: solvent A: IPA  
           solvent B: Water  
           +0.1% TFA  
 Gradient: linear

FIGURE 5B

FIGURE 5C

FIGURE 5D

## Naloxone Antagonism of Cetyl-PEG<sub>2</sub>-Enkephalin-Induced Analgesia

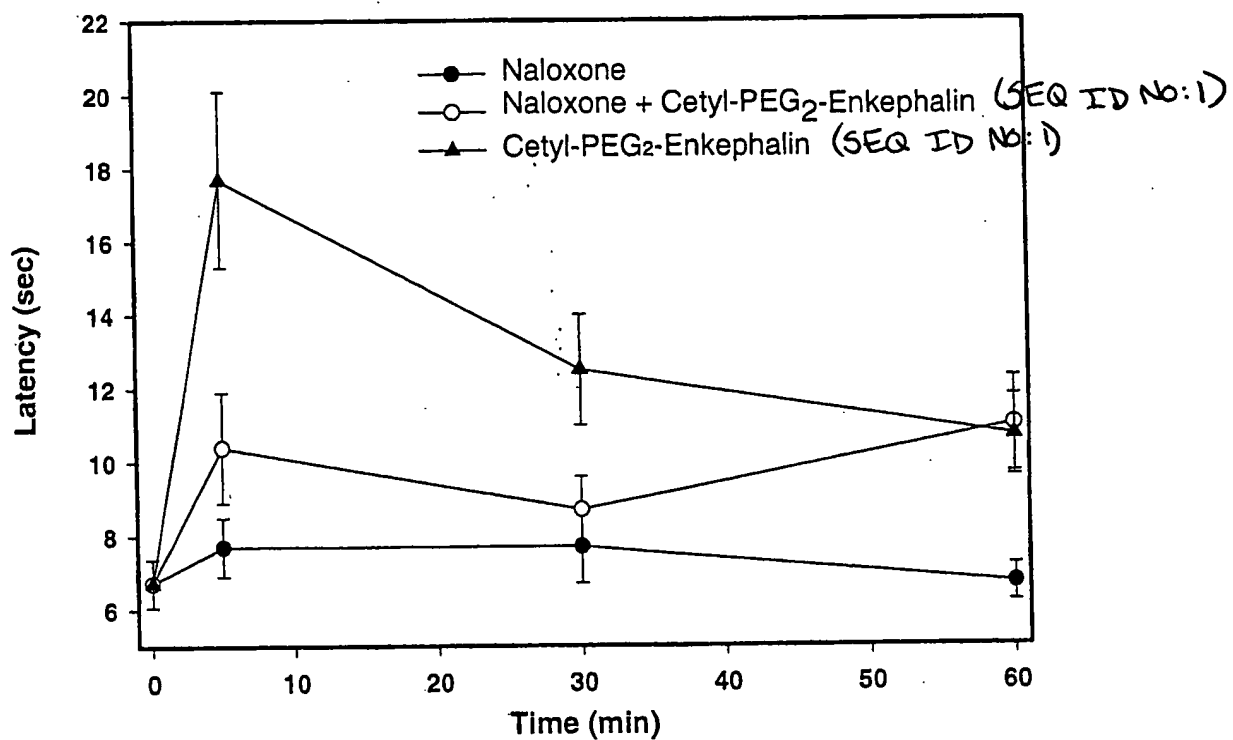
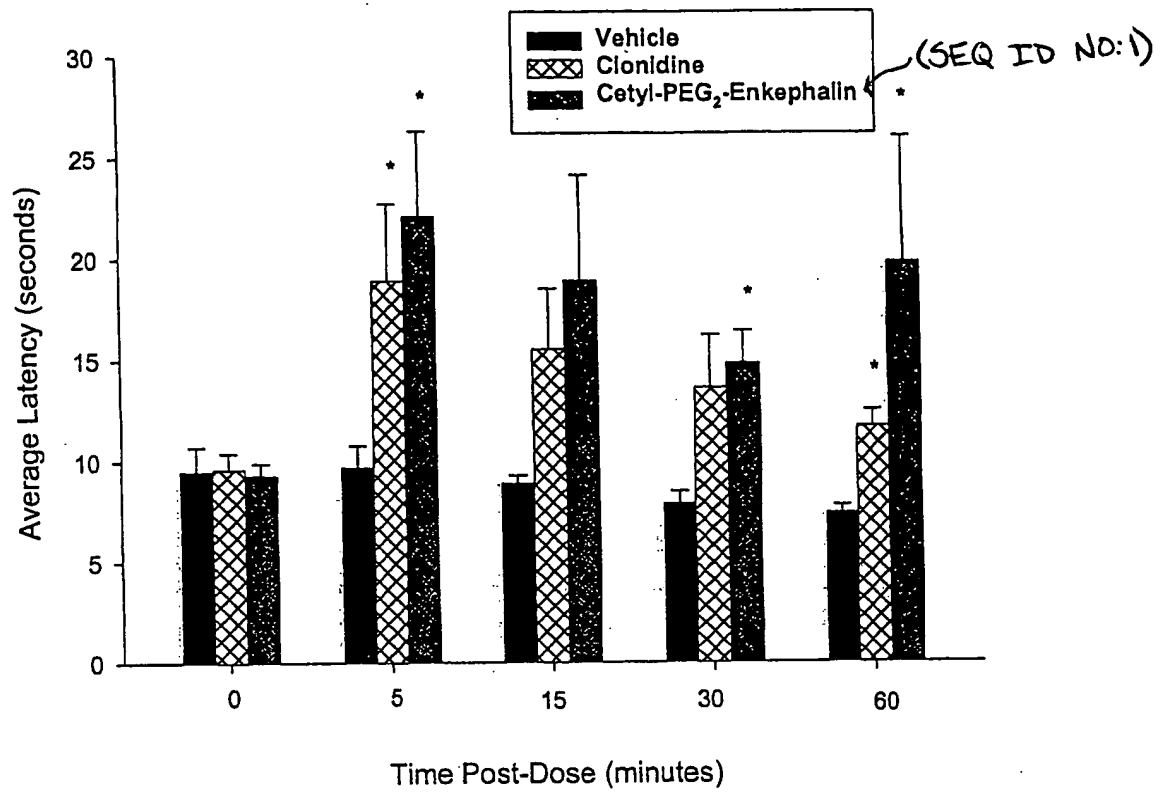


FIGURE 6

**Analgesic Effect of a 5 mg/kg IV Dose of Cetyl-PEG<sub>2</sub>-Enkephalin (SEQ ID No:1)  
Monoconjugate in the Rat Hot-Plate Assay**



**FIGURE 7**

COMPARISON OF $\mu$ -RECEPTOR BINDING AFFINITY OF ENKEPHALIN CONJUGATES		
DRUG OR CONJUGATE	DETAILED STRUCTURE	% SPECIFIC BINDING*
Naloxone	Naloxone	100
Enkephalin	Met-Enkephalin -Lys (5EQ ID No:49)	67
Cetyl-ENK	Cetyl-PEG <sub>2</sub> -ENK (5EQ ID No:1)	100
Chol-ENK	Cholesterol-PEG <sub>3</sub> -ENK (5EQ ID No:1)	95
DHA-ENK	DHA-PEG <sub>2</sub> -ENK (5EQ ID No:1)	63
Palm-ENK	Palmitate-PEG <sub>3</sub> -ENK (5EQ ID No:1)	76
Cetyl-TEG-ENK	Cetyl-PEG <sub>3</sub> -ENK (5EQ ID No:1)	100

\*Data are based on percent inhibition at a concentration of 100nM. The radioligand was DAMGO ([D-Ala<sup>2</sup>,N-Me-Phe-Gly<sup>5</sup>-ol]enkephalin) and naloxone served as the reference.

FIGURE 8